Glucocorticoid-Induced Diabetes: Potential Role for Incretin-Based Therapies

van Genugten, R.E.

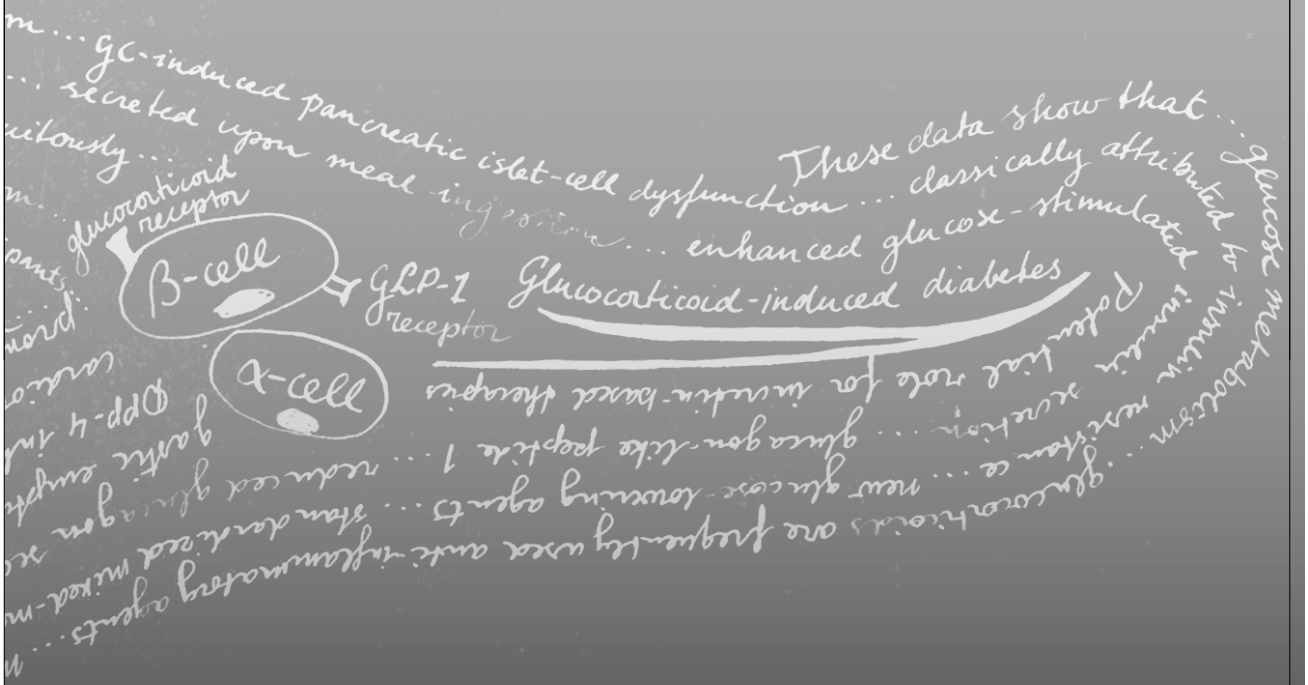
2013

document version
Publisher's PDF, also known as Version of record

CHAPTER 4

Glucagon-Like Peptide-1 Receptor Agonist Treatment Prevents Glucocorticoid-Induced Glucose Intolerance and Islet-Cell Dysfunction in Humans

Renate E. van Genugten*, Daniël H. van Raalte*, Margot M.L. Linssen, D. Margriet Ouwens, Michaela Diamant
*Both authors contributed equally



Diabetes Care. 2011 Feb;34(2):412-7.

ABSTRACT

Objective

Glucocorticoids (GCs) are regarded as diabetogenic since they impair insulin sensitivity and islet-cell function. In this study we assessed whether treatment with the glucagon-like peptide receptor agonist (GLP-1 RA) exenatide (EXE) could prevent GC-induced glucose intolerance.

Research Design and Methods

A randomized, placebo-controlled, double blind, crossover study in 8 healthy males (age: 23.5 (20.0-28.3) years; BMI: 26.4 (24.3-28.0) kg/m^2) was conducted. Participants received 3 therapeutic regimens for 2 consecutive days, i.e. (1) 80 mg oral prednisolone (PRED) QD and intravenous (IV.) EXE infusion (PRED+EXE), (2) 80 mg oral PRED QD and IV. saline infusion (PRED+SAL), and (3) oral placebo-PRED QD and IV. saline infusion (PLB+SAL). On day 1, glucose tolerance was assessed during a meal challenge test. On day 2, participants underwent a clamp procedure to measure insulin secretion and insulin sensitivity.

Results

PRED+SAL treatment increased postprandial glucose levels (vs. PLB+SAL, *P*= 0.012), which was prevented by concomitant EXE (vs. PLB+SAL, *P* = NS). EXE reduced PRED-induced hyperglucagonemia during the meal challenge (*P* = 0.018) and decreased gastric emptying (vs. PRED+SAL, *P* = 0.028; vs. PLB+SAL, *P* = 0.046). PRED+SAL decreased first-phase glucose-stimulated and arginine-stimulated C-peptide secretion (vs. PLB+SAL, *P* = 0.017 and *P* = 0.05 respectively), while PRED+EXE improved first-phase and second-phase glucose-stimulated, as well as arginine-stimulated C-peptide secretion (vs. PLB+SAL; *P* = 0.017, 0.012 and 0.093 respectively).

Conclusions

The GLP-1 RA EXE prevented PRED-induced glucose intolerance and islet-cell dysfunction in healthy humans. Incretin-based therapies should be explored as a potential strategy to prevent steroid diabetes.

Clinical Trial Registration Number

NCT00744224

Glucocorticoids (GCs) are diabetogenic agents since they reduce insulin sensitivity (1), impair alpha-cell function (2), and, according to more recent findings, impair beta-cell function (3-4). As such, chronic use of GCs was associated with odds ratios between 1.4 and 2.3 to develop diabetes (5-7). Loss of glycemic control during GC use is particularly due to impaired postprandial glucose metabolism, while fasting plasma glucose (FPG) levels are usually only mildly elevated (4,7). Although the exact prevalence of steroid-related diabetes is unknown, the widespread use of GCs indicates that it may represent a major clinical problem worldwide.

When initiating GC therapy in current clinical practice, preventive pharmacological measures are taken to prevent some of the GC-related side-effects, most notably osteoporosis and peptic ulcer disease (8,9). Interestingly, in spite of the above-mentioned highly-prevalent occurrence of steroid-diabetes, to date, no strategies are undertaken to prevent the adverse metabolic effects of GC treatment. Previous studies showed that metformin and the thiazolidinedione (TZD) pioglitazone were unable to mitigate the effects of GCs on glucose tolerance (10), while the TZD troglitazone prevented GC-induced hyperglycemia by enhancing GC clearance (10,11). Due to liver toxicity, however, troglitazone is no longer available for treatment in humans.

The gut hormone glucagon-like peptide (GLP)-1 and synthetic dipeptidyl-peptidase (DPP)-4 resistant GLP-1 receptor agonists (GLP-1 RA), such as exenatide (EXE), lower blood glucose by, glucose-dependently, enhancing insulin secretion and production and inhibiting glucagon secretion, and by slowing down gastric emptying (12). One year of EXE treatment was shown to improve clamp-measured beta-cell function in patients with type 2 diabetes mellitus (T2DM) (13) Recently, the GLP-1 RA exendin-4 was shown to prevent GC-induced beta-cell apoptosis *in vitro* (14). In a single patient with Cushing's disease, GLP-1 infusion was as effective in lowering blood glucose levels as compared to patients with 'typical' T2DM (15). Finally, GLP-1 infusion effectively reduced stress hyperglycemia in patients undergoing coronary artery bypass graft (CABG) (16).

Given these beneficial effects of GLP-1 RA treatment, and the pathophysiologic defects underlying GC-induced glucose intolerance and diabetes, we aimed to assess whether IV. infusion of the GLP-1 RA EXE could prevent the acute adverse effects of prednisolone (PRED) treatment on glucose metabolism, islet-cell function and insulin sensitivity in healthy normoglycemic individuals.

RESEARCH DESIGN AND METHODS

Participants

Eight healthy males were recruited via local advertisements. Inclusion criteria included age = 18-35 years, body mass index (BMI) = 22.0-28.0 kg/m^2, good physical health (determined by medical history, physical examination and screening blood tests) and normoglycemia as defined by FPG <

5.6 mmol/L and 2-hour glucose < 7.8 mmol/L following a 75g oral glucose tolerance test (OGTT), performed at screening visit. Exclusion criteria were the presence of any disease, use of any medication, first-degree relative with type 2 diabetes, smoking, shift work, a history of GC use and recent changes in weight or physical activity. The study was approved by an independent ethics committee and the study was conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent before participation.

Experimental Design

The study was a randomized, placebo-controlled, double blind crossover study. Following assessment of eligibility, participants received for two consecutive days, in random order: (1) 80 mg oral PRED QD and IV. EXE infusion (PRED+EXE), (2) 80 mg oral PRED QD and IV. saline infusion (PRED+SAL), and (3) oral placebo-PRED QD and IV. saline infusion (PLB+SAL). On day 1, a standardized meal challenge was performed (Supplemental Figure 1A) and on day 2, participants underwent a combined clamp procedure, starting with a hyperinsulinemic-euglycemic clamp, followed by a hyperglycemic clamp with subsequent arginine stimulation (Supplemental Figure 1B) (13). The three 2-day treatment blocks were separated by at least 4 weeks. Participants were instructed to refrain from intense physical activity 2 days before each treatment block.

The primary endpoint was the 4-hour glucose area under the curve (AUC_G) during the meal challenge test. From previous studies, we expected an increase in AUC_G of 400 mmol/L•240min following PRED treatment (4). A sample size of 8 would provide 80% power to detect a significant reduction of 50% by EXE of PRED-induced augmentation of AUC_G. Secondary endpoints included first-phase and second-phase C-peptide incremental area under the curve ($iAUC_{CP}$), and arginine-stimulated C-peptide secretion (ASI- $iAUC_{CP}$) during the hyperglycemic clamp.

Standardized Meal Challenge

On day 1 of each treatment block, participants underwent a standardized meal challenge test following an overnight fast of minimally 10 hours. The meal contained 905 kcal (50 g fat, 75 g carbohydrates, 35 g protein) and 1g liquid acetaminophen to estimate gastric emptying rates. Samples for determination of glucose, insulin, C-peptide, glucagon, acetaminophen and EXE were obtained at times -120, -60, -30, 0, 10, 20, 30, 60, 90, 120, 150, 180 and 240 min, with the meal beginning immediately after the time 0 sample and consumed within 15 min. Eighty mg oral PRED or PLB was ingested 2 hours before meal consumption, and IV. EXE or SAL infusion started 60 min before the meal at an infusion rate of 40 ng/min for 30 min, and was decreased to 20 ng/min for the remainder of the test (Supplemental Figure 1A).

Hyperinsulinemic-Euglycemic Clamp and Hyperglycemic Clamp

On day 2 of each block**,** a combined hyperinsulinemic-euglycemic and hyperglycemic clamp procedure was done. After an overnight fast, an indwelling cannula was inserted into an antecubital vein for infusion of glucose and insulin. To obtain arterialized venous blood samples, a retrograde cannula was inserted in a contralateral wrist vein and maintained in a heated box at 50°C. Insulin was infused at a rate of 40 mU/m^2•min for 120 min, plasma glucose was kept at 5 mM by a variable infusion of 20% glucose. The hyperglycemic clamp was started 90 min after cessation of exogenous insulin infusion. Plasma glucose concentration was then raised to 10 mM by a body weight-adjusted intravenous bolus of 20% glucose and a variable 20% glucose infusion was adjusted to maintain the targeted glucose level. After 80 min hyperglycemia, an intravenous bolus of 5 g arginine (dissolved in 50 mL) was given over 45 seconds, and the glucose level was maintained at 10 mM for 30 min. Eighty mg oral PRED or PLB was administered 2 hours before initiation of hyperinsulinemic-euglycemic clamp. IV. EXE or SAL infusion was started 60 min before the start of the hyperglycemic clamp at an infusion rate of 40 ng/min for 30 min, and was decreased to 20 ng/min for the rest of the hyperglycemic clamp (Supplemental Figure 1B). Note that EXE was not infused during the hyperinsulinemic-euglycemic clamp, since, in a pilot study, EXE strongly induced insulin secretion at a glucose level of 5 mM. The hyperinsulinemic-euglycemic clamp was performed in order to be able to calculate the disposition index (see below).

Study Medication

PRED tablets were purchased from Pfizer AB (Sollentuna, Sweden) and PLB tablets were obtained from Xendo Drug Development (Groningen, The Netherlands). Tablets were capsulated in order to allow the treatment to be blinded (4). Commercially available EXE was purchased (Pharmacy VU University Medical Center) and diluted in saline containing 1% human serum albumin, forming a colorless solution. IV. administration of EXE was chosen in order to be able to gain steady-state EXE levels within a short period of time. The plasma EXE target level was 100 pg/ml, which demonstrated good efficacy and tolerability during previous infusion experiments (17).

Analytical Determinations

Blood glucose concentrations were measured using an YSI 2300 STAT Plus analyzer (YSI, Yellow Springs, OH). Insulin and C-peptide levels were determined using an immunometric assay (Advia, Centaur, Siemens Medical Solutions Diagnostics, USA). Glucagon (Linco Research, St. Louis, USA) and acetaminophen (Abbott Laboratories, IL, USA) concentrations were determined by radioimmuno assay. Exenatide levels were determined by an immunoenzymetric assay as described previously (Amylin, San Diego, CA) (17). Body fat percentage was estimated by bioelectrical impedance analysis (BF-906, Maltron International, UK).

Data Analyses

Absolute area under the curves (AUCs) for glucose, insulin, C-peptide, glucagon and acetaminophen were calculated during the 4-hr meal challenge using the trapezoid method. The Matsuda whole-body insulin sensitivity index was calculated from the meal challenge. Whole-body insulin sensitivity as obtained from the hyperinsulinemic-euglycemic clamp was quantified by the M-value, calculated between min 90-120 during steady-state insulin concentrations. $iAUC_{CP}$ for the first-phase (min. 0-10), second-phase (min. 10-80) and ASI-$iAUC_{CP}$ (min. 80-110) were calculated during the hyperglycemic clamp using the trapezoid method. The disposition index (DI) from the clamp tests was calculated by the C-peptide iAUC multiplied by the M-value.

Statistical Analyses

Data are presented as mean values ± standard error of the mean (SEM) or, in case of skewed distribution, as median (interquartile range). Between-block differences were tested non-parametrically with Friedman's Test and, in case of a significant result, further analyzed using Wilcoxon Signed Ranks Test. All statistical analyses were run on SPSS (SPSS, Chicago, IL, USA). A $P < 0.05$ was considered statistically significant.

RESULTS

Subject Characteristics

8 healthy Caucasian males were included (median (interquartile range)): age = 23.5 (20.0-28.3) years; BMI = 25.8 (23.2-27.7) kg/m^2; waist = 91 (82-95) cm; body fat = 21 (15-26) %; FPG = 5.0 (4.8-5.3) mmol/L; triglycerides = 1.1 (0.7-1.5) mmol/L; systolic blood pressure = 119 (117-125) mmHg; diastolic blood pressure = 77 (72-82) mmHg.

Standardized Meal Challenge

PRED+SAL treatment increased AUC_G as compared to PLB+SAL ($P = 0.012$), which was prevented by concomitant EXE administration (Table 1, Figure 1A). AUC for insulin (AUC_I) was not decreased by PRED+SAL, although AUC for C-peptide (AUC_{CP}) tended to be lower ($P = 0.07$). PRED+EXE significantly decreased both AUC_I and AUC_{CP}. (Table 1, Figure 1B+C). PRED+SAL non-significantly increased glucagon secretion compared to PLB+SAL ($P = 0.09$), which was mitigated by EXE treatment (Figure 1D). EXE significantly decreased AUC for acetaminophen (AUC_{ACET}) compared to both PLB and PRED, compatible with its gastric emptying slowing effects. The Matsuda whole-body insulin sensitivity index increased during EXE treatment both vs. PLB and PRED (Table 1).

Table 1. Results from the Standardized Meal Challenge.

	PLB+SAL N = 8	PRED+SAL N = 8	PRED+EXE N = 8	P-Value PLB+SAL vs. PRED+SAL	PLB+SAL vs. PRED+EXE	PRED+SAL vs. PRED+EXE
AUC_G (mmol/L•240min)	1199 (1043-1248)	1335 (1254-1501)	1247 (1156-1260)	0.012	0.263	0.025
AUC_I (nmol/L•240min)	66.2 (35.2-81.0)	52.5 (27.2-70.2)	32.2 (22.8-40.1)	0.263	0.017	0.017
AUC_{CP} (nmol/L•240min)	354 (212-382)	270 (191-328)	203 (184-221)	0.069	0.017	0.017
AUC_{GCG} (pmol/L•240min)	2845 (1941-2965)	3085 (2859-3633)	2232 (1850-3149)	0.091	0.499	0.018
AUC_{ACET} (mg/L•240min)	1272 (1002-1485)	1449 (1243-1542)	940 (801-1039)	0.6	0.028	0.046
Matsuda index (No dimension)	22.6 (13.0-39.4)	20.4 (16.1-41.2)	36.8 (26.9-50.0)	0.575	0.025	0.012

Abbreviations: AUC: area under the curve; AUC_{ACET}: acetaminophen AUC; AUC_{CP}: C-peptide AUC; AUC_{GCG}: glucagon AUC; AUC_G: glucose AUC; AUC_I: insulin AUC; EXE: exenatide; PLB: placebo; PRED: prednisolone; SAL: saline.

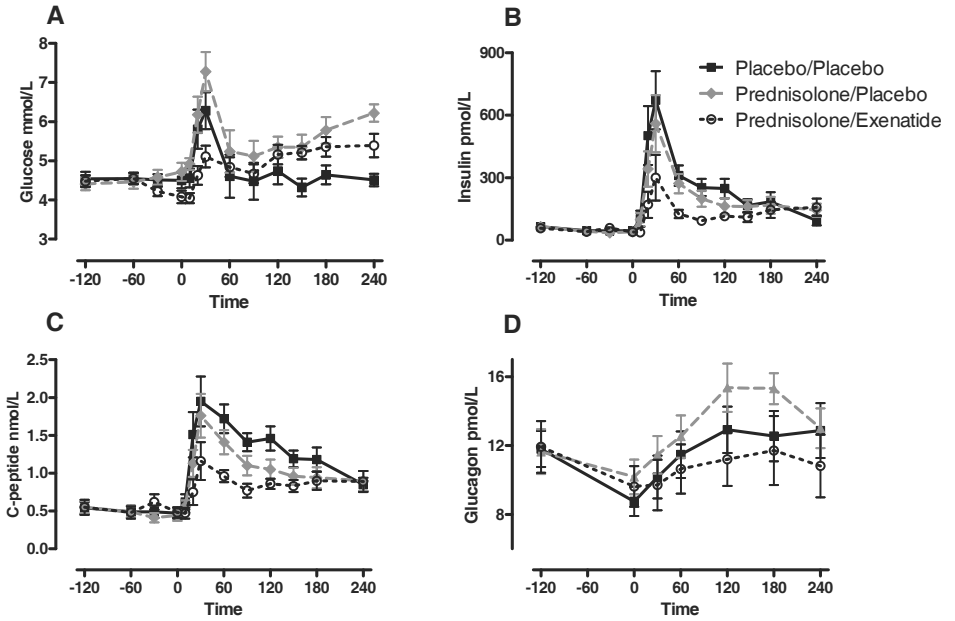


Figure 1. **The effect of PRED with or without concomitant EXE infusion on plasma glucose (A), insulin (B), C-peptide (C) and glucagon (D) levels during the meal challenge.** PRED increased AUC_G, which was prevented by EXE (A), despite lower insulin and C-peptide levels (B+C). EXE infusion reduced postprandial glucagon levels as compared PRED (D). Mean ± SEM are shown. Black solid line with closed squares: PLB+SAL; Grey intersected line with closed circles: PRED+SAL; Black dotted line with open circles: PRED+EXE.

COMBINED CLAMP PROCEDURE

C-peptide Secretion, Hyperglycemic Clamp

PRED decreased first-phase $iAUC_{CP}$ and ASI-$iAUC_{CP}$ (vs. PLB+SAL; $P = 0.017$ and $P = 0.05$ respectively), but did not affect second-phase $iAUC_{CP}$ (Table 2). EXE restored PRED-induced reductions in first-phase $iAUC_{CP}$ and ASI-$iAUC_{CP}$ and significantly improved C-peptide secretion during the entire clamp, both compared to PRED+SAL and PLB+SAL (Table 2, Figure 2A). Insulin iAUC results were not different from the C-peptide iAUC results (Figure 2B).

Table 2. Results from the Hyperglycemic Clamp

				P-Value		
	PLB+SAL N = 8	PRED+SAL N = 8	PRED+EXE N = 8	PLB+SAL vs. PRED+SAL	PLB+SAL vs. PRED+EXE	PRED+SAL vs. PRED+EXE
1st $iAUC_{CP}$ (nmol•min/L)	6 (4-8)	4 (2-6)	10 (8-13)	0.017	0.017	0.012
2nd $iAUC_{CP}$ (nmol•min/L)	30 (17-48)	26 (18-53)	111 (63-117)	0.779	0.012	0.012
1st+2nd $iAUC_{CP}$ (nmol•min/L)	83 (79-107)	71 (41-100)	201 (160-249)	0.208	0.012	0.012
ASI $iAUC_{CP}$ (nmol•min/L)	26 (24-34)	18 (17-29)	37 (28-43)	0.05	0.093	0.012

Abbreviations: ASI: arginine-stimulated; $iAUC_{CP}$: incremental area under the curve for C-peptide. EXE: exenatide; PLB: placebo; PRED: prednisolone; SAL: saline.

Insulin Sensitivity, Euglycemic Clamp

Insulin levels reached steady-state during min 90-120 of the euglycemic clamp, averaging 431 ± 62 (PLB+SAL), 418 ± 73 (PRED+SAL) and 422 ± 57 pM (PRED+EXE). PRED acutely decreased the M-value obtained from the euglycemic clamp by 20% ($P = 0.018$) (Figure 2C). Adjustment of the M-value by insulin levels during the steady-state part of the clamp (M/I) did not affect the results (data not shown). Note that the effects of EXE on whole-body insulin sensitivity were not assessed during the euglycemic clamp, EXE was administered during the hyperglycemic clamp only.

Disposition Index

PRED+SAL decreased the DI from T = 0-80 min of the hyperglycemic clamp (combined first- and second-phase; $P = 0.012$) and the DI from T = 80-110 min of the hyperglycemic clamp (arginine stimulation; $P = 0.012$). The PRED-induced decrease in DI was fully restored by concomitant EXE infusion, and EXE significantly improved the DI as compared to PLB+SAL from T = 0-80 min (Figure 2D+E).

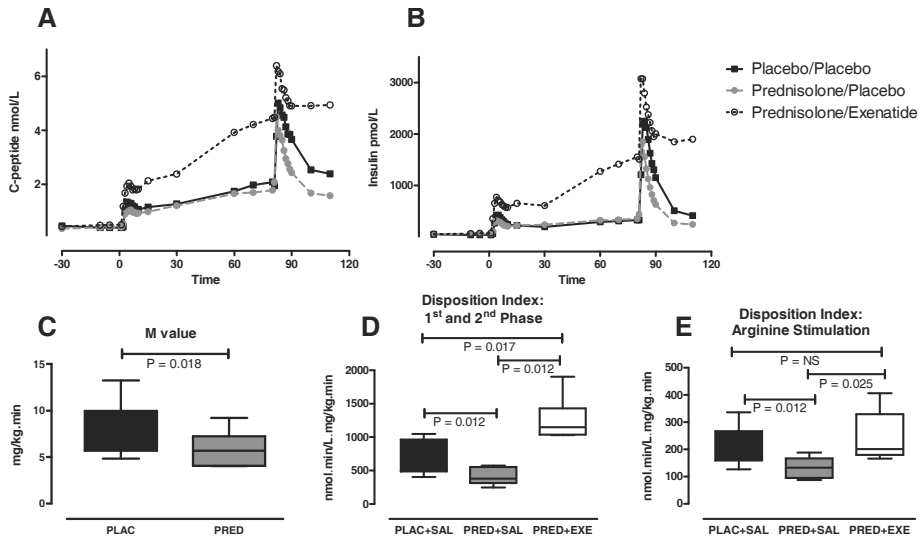


Figure 2. **The effect of PRED with or without concomitant EXE infusion on C-peptide (A) and insulin (B) concentrations during the hyperglycemic clamp.** PRED+SAL decreased first-phase (min 0-10) and arginine-stimulated C-peptide secretion (min 80-110), which were completely restored and improved by concomitant EXE administration (Black solid line with closed squares: PLB+SAL; Grey intersected line with closed circles: PRED+SAL; Black dotted line with open circles: PRED+EXE). Panel C depicts PRED-induced changes in M-value during the euglycemic clamp. PRED reduced combined first- and second-phase (min 0-80) disposition index (DI) (panel D) and arginine-stimulated (min 80-110) DI (panel E), which was restored and improved by EXE (Box-and-Whisker plots (min-max) are shown).

EXE Plasma Levels/Adverse Effects

Mean EXE plasma levels equaled 65 ± 4 pg/mL between T = 0 and T = 240 of the meal challenge (Supplemental Figure 2A) and 80 ± 4 pg/mL between T = -30 and T = 110 of the hyperglycemic clamp (Supplemental Figure 2B). No adverse effects of either PRED or EXE treatment were experienced by the participants during the meal or clamp procedure.

DISCUSSION

GCs are known to impair glucose metabolism by inducing insulin resistance and, more recently, beta-cell dysfunction (1,4). This study is the first to demonstrate that treatment with the GLP-1RA EXE prevents PRED-induced glucose intolerance as assessed by a standardized meal challenge test. During the hyperglycemic clamp, EXE infusion restored PRED-induced impairment of beta-cell function variables, and even significantly improved a number of these variables relative to the control situation. In contrast to the findings observed during the clamp procedure, EXE treatment given during the meal challenge, improved glucose tolerance but resulted in decreased insulin plasma

levels. This observation is in line with a previous study in healthy individuals in which subcutaneous EXE treatment reduced postprandial glucose excursions despite significantly lower insulin levels (18). The glucose-lowering effects of EXE were attributed to decreased glucagon secretion and gastric emptying and, due to its glucose-dependent mode of action, EXE did not further stimulate insulin secretion in the presence of normoglycemia (18). In our study, we similarly found reduced postprandial glucagon secretion and gastric emptying following EXE treatment. Studies employing stable isotope techniques have demonstrated that EXE may also reduce hepatic glucose output and increase whole-body glucose disposal in the postprandial state, independent of its more established effects on islet hormone secretion and gastric emptying (19,20). In our study, EXE improved whole-body insulin sensitivity during the meal challenge as estimated by the Matsuda index, however, reduced glucose appearance due to decreased gastric emptying seemed primarily responsible for improving glucose tolerance. We did not assess the effects of EXE on whole-body insulin sensitivity during the hyperinsulinemic-euglycemic clamp for previous mentioned reasons.

In this proof-of-principle study, both treatment regimens were administered for a short period of time: i.e. for two consecutive days per study block. Although the acute metabolic effects of both PRED and EXE may to some extent differ from their effects following prolonged administration, it provides a good model to study the effects of each of both drugs as well as their interaction. IV. EXE infusion was able to prevent the acute adverse effects of PRED on glucose tolerance, and additional benefits from EXE treatment may be expected when both compounds are administered for a more prolonged time period. Chronic GC use is associated with increased appetite, significant weight gain, increased visceral fat mass, altered secretion of adipocytokines and dyslipidemia (1), all of which contribute to the adverse effects of GCs on glucose metabolism. In clinical studies in T2DM patients, chronic EXE treatment was shown to reduce appetite, resulting in substantial weight loss, decrease in truncal fat mass, and increased secretion of adiponectin (13,21,22). Also, EXE improved postprandial dyslipidemia (23). The strong reduction of postprandial glucose levels by EXE, rather than a pronounced effect on FPG (13), matches the profile of GC-induced hyperglycemia, which is predominantly present during the day (7).

During the hyperglycemic clamp experiments, pharmacological concentrations of EXE were able to restore PRED-induced changes in beta-cell function, including first-phase and ASI C-peptide secretion and DI calculated for the entire hyperglycemic clamp. GC exposure was demonstrated to impair various pathways in the beta-cell *in vitro*. These include both steps in the uptake and metabolism of glucose, but GCs also affected distal pathways in the insulin exocytosis process, resulting in impaired insulin secretion in response to different secretagogues (3;4). Since EXE was able to restore insulin secretion, one may speculate that GCs do not block the pathways mediating GLP-1 action on beta cells. However, it was very recently reported that a two-week treatment with oral PRED reduced

the insulinotropic effects of endogenous GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) (24).

A limitation of our study is that we treated healthy subjects with GCs, while GCs are prescribed to treat acute and chronic inflammatory, as well as autoimmune diseases. Chronic inflammation is also associated with whole-body insulin resistance and beta-cell dysfunction, as was recently reported in patients with rheumatoid arthritis (25). Therefore, the complex interrelationship between inflammation, GC and GLP-1RA treatment needs do be studied prospectively in relevant patient populations.

The plasma levels of EXE reached with our infusion protocol were lower than those usually obtained following subcutaneous injection of EXE 10 mg BID, i.e. the recommended dose for the current treatment in T2DM. Although good efficacy was demonstrated by current plasma EXE levels, the full potential of GLP-1 RA treatment to prevent PRED-induced glucose intolerance may be fully unveiled in clinical studies administering EXE at the usual dose.

Taken together, this study provides evidence that the GLP-1 RA EXE may prevent PRED-induced glucose intolerance and restore islet-cell functional balance. Long-term studies in relevant populations should explore the potential of GLP-1 RA treatment as a novel strategy to prevent steroid diabetes.

ACKNOWLEDGMENTS

The authors express their gratitude to Mark Fineman (Amylin Pharmaceuticals) for the determination of EXE plasma levels.´,

REFERENCES

1. van Raalte DH, Ouwens DM, Diamant M. Novel insights into glucocorticoid-mediated diabetogenic effects: towards expansion of therapeutic options? *Eur J Clin Invest* 2009;39:81-93
2. Wise JK, Hendler R, Felig P. Influence of glucocorticoids on glucagon secretion and plasma amino acid concentrations in man. *J Clin Invest* 1973;52:2774-2782
3. Lambillotte C, Gilon P, Henquin JC. Direct glucocorticoid inhibition of insulin secretion. An in vitro study of dexamethasone effects in mouse islets. *J Clin Invest* 1997;99:414-423
4. van Raalte DH, Nofrate V, Bunck MC, van Iersel T, Elassaiss SJ, Nassander UK, Heine RJ, Mari A, Dokter WH, Diamant M. Acute and 2-week exposure to prednisolone impair different aspects of beta-cell function in healthy men. *Eur J Endocrinol* 2010;162:729-735.
5. Gulliford MC, Charlton J, Latinovic R. Risk of diabetes associated with prescribed glucocorticoids in a large population. *Diabetes Care* 2006;29:2728-2729
6. Gurwitz JH, Bohn RL, Glynn RJ, Monane M, Mogun H, Avorn J. Glucocorticoids and the risk for initiation of hypoglycemic therapy. *Arch Intern Med* 1994;154:97-101
7. Clore JN, Thurby-Hay L. Glucocorticoid-induced hyperglycemia. *Endocr Pract* 2009;15:469-474
8. Compston J. Management of glucocorticoid-induced osteoporosis. *Nat Rev Rheumatol* 2010;6:82-88
9. Trikudanathan S, McMahon GT. Optimum management of glucocorticoid-treated patients. *Nat Clin Pract Endocrinol Metab* 2008;4:262-271
10. Morita H, Oki Y, Ito T, Ohishi H, Suzuki S, Nakamura H. Administration of troglitazone, but not pioglitazone, reduces insulin resistance caused by short-term dexamethasone (DXM) treatment by accelerating the metabolism of DXM. *Diabetes Care* 2001;24:788-789
11. Willi SM, Kennedy A, Wallace P, Ganaway E, Rogers NL, Garvey WT. Troglitazone antagonizes metabolic effects of glucocorticoids in humans: effects on glucose tolerance, insulin sensitivity, suppression of free fatty acids, and leptin. *Diabetes* 2002;51:2895-2902
12. Baggio LL, Drucker DJ. Biology of incretins: GLP-1 and GIP. *Gastroenterology* 2007;132:2131-2157
13. Bunck MC, Diamant M, Corner A, Eliasson B, Malloy JL, Shaginian RM, Deng W, Kendall DM, Taskinen MR, Smith U, Yki-Jarvinen H, Heine RJ. One-year treatment with exenatide improves beta-cell function, compared with insulin glargine, in metformin-treated type 2 diabetic patients: a randomized, controlled trial. *Diabetes Care* 2009;32:762-768
14. Ranta F, Avram D, Berchtold S, Dufer M, Drews G, Lang F, Ullrich S. Dexamethasone induces cell death in insulin-secreting cells, an effect reversed by exendin-4. *Diabetes* 2006 55:1380-1390
15. Ritzel RA, Kleine N, Holst JJ, Willms B, Schmiegel W, Nauck MA Preserved GLP-1 effects in a diabetic patient with Cushing's disease. *Exp Clin Endocrinol Diabetes* 2007;115:146-150
16. Sokos GG, Bolukoglu H, German J, Hentosz T, Magovern GJ Jr, Maher TD, Dean DA, Bailey SH, Marrone G, Benckart DH, Elahi D, Shannon RP. Effect of glucagon-like peptide-1 (GLP-1) on glycemic control and left ventricular function in patients undergoing coronary artery bypass grafting. *Am J Cardiol* 2007;100:824-829.
17. Degn KB, Brock B, Juhl CB, Djurhuus CB, Grubert J, Kim D, Han J, Taylor K, Fineman M, Schmitz O. Effect of intravenous infusion of exenatide (synthetic exendin-4) on glucose-dependent insulin secretion and counterregulation during hypoglycemia. *Diabetes* 2004;53:2397-2403

18. Kolterman OG, Buse JB, Fineman MS, Gaines E, Heintz S, Bicsak TA, Taylor K, Kim D, Aisporna M, Wang Y, Baron AD. Synthetic exendin-4 (exenatide) significantly reduces postprandial and fasting plasma glucose in subjects with type 2 diabetes. *J Clin Endocrinol Metab* 2003;88:3082-3089

19. Cervera A, Wajcberg E, Sriwijitkamol A, Fernandez M, Zuo P, Triplitt C, Musi N, Defronzo RA, Cersosimo E. Mechanism of action of exenatide to reduce postprandial hyperglycemia in type 2 diabetes. *Am J Physiol Endocrinol Metab* 2008;294:E846-E852

20. Zheng D, Ionut V, Mooradian V, Stefanovski D, Bergman RN. Exenatide sensitizes insulin-mediated whole-body glucose disposal and promotes uptake of exogenous glucose by the liver. *Diabetes* 2009;58:352-359

21. Bunck MC, Diamant M, Eliasson B, Corner A, Shaginian RM, Heine RJ, Taskinen MR, Yki-Jarvinen H, Smith U. Exenatide Affected Circulating Cardiovascular Risk Biomarkers Independently Of Changes In Body Composition. *Diabetes Care* 2010;33:1734-1737.

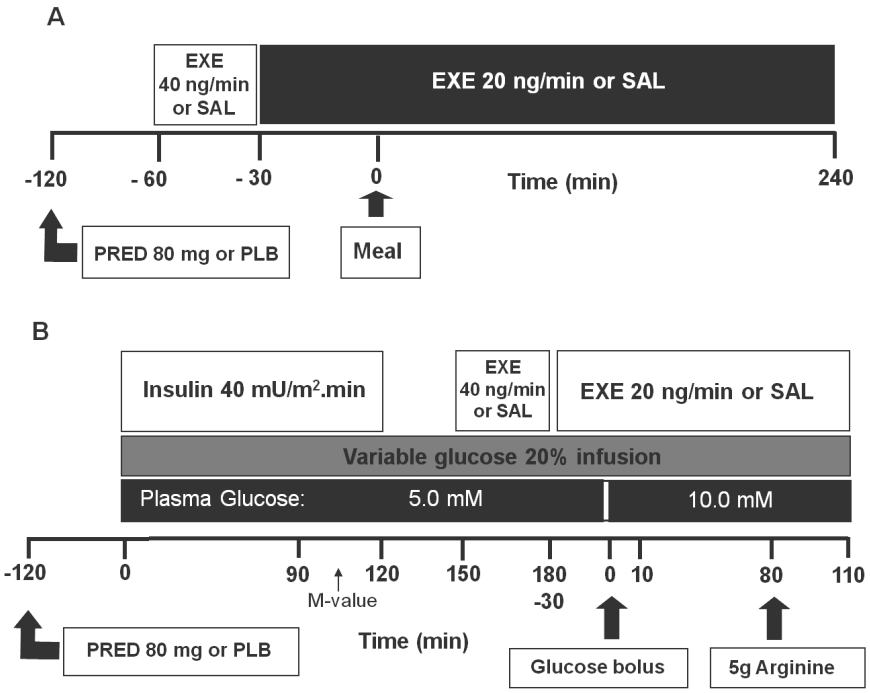
22. Klonoff DC, Buse JB, Nielsen LL, Guan X, Bowlus CL, Holcombe JH, Wintle ME, Maggs DG. Exenatide effects on diabetes, obesity, cardiovascular risk factors and hepatic biomarkers in patients with type 2 diabetes treated for at least 3 years. *Curr Med Res Opin* 2008;24:275-286

23. Bunck MC, Corner A, Eliasson B, Heine RJ, Shaginian RM, Wu Y, Yan P, Smith U, Yki-Jarvinen H, Diamant M, Taskinen MR. One-year treatment with exenatide vs. insulin glargine: effects on postprandial glycemia, lipid profiles, and oxidative stress. *Atherosclerosis* 2010 [Epub ahead of print]

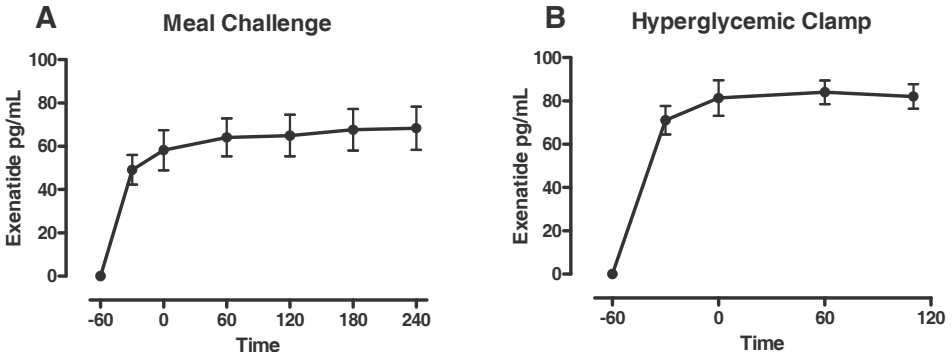
24. Hansen KB, Vilsboll T, Bagger JI, Holst JJ, Knop FK. Reduced Glucose Tolerance and Insulin Resistance Induced by Steroid Treatment, Relative Physical Inactivity, and High-Calorie Diet Impairs the Incretin Effect in Healthy Subjects. *J Clin Endocrinol Metab* 2010;95:3309-3317

25. Dessein PH, Joffe BI. Insulin resistance and impaired beta cell function in rheumatoid arthritis. *Arthritis Rheum* 2006;54:2765-2775

SUPPLEMENTAL FIGURES



Supplemental Figure 1. Overview of the meal challenge test (A) and the combined hyperinsulinemic-euglycemic and hyperglycemic clamp procedure (B). Note that EXE was infused during the hyperglycemic clamp, but not during the euglycemic clamp. Abbreviations: EXE: exenatide; PRED: prednisolone.



Supplemental Figure 2. Exenatide plasma levels during the meal challenge (A) and hyperglycemic clamp test (B). EXE plasma levels equaled 65 ± 4 pg/mL between T = 0 and T = 240 of the meal challenge (A) and 80 ± 4 pg/mL between T = -30 and T = 110 of the hyperglycemic clamp (B). Mean ± SEM are depicted.